

Supplemental Information

Specialized mechanosensory nociceptors mediating rapid responses to hair-pull

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Supplemental Information

Figure S1 **Related to Figure 1**

Figure S2 **Related to Figure 2 and Figure 3**

Figure S3 **Related to Figure 2 and Figure 3**

Figure S4 **Related to Figure 3 and Figure 4**

Figure S5 **Related to Figure 3 and Figure 4**

Figure S6 **Related to Figure 3**

Figure S7 **Related to Figure 3 and Figure 4**

Figure S1. Related to Figure 1; Broad reporter expression in Trpv1-Cre mice. (A)

Immunostaining of a trigeminal ganglion section from a Trpv1^{lin}-tdT mouse showing tdTomato (red) is found in sensory neurons with heterogeneous cell diameters. Only the largest neurons are stained by NF200 (green). **(B)** Section of hairy skin from a Trpv1^{lin}-tdT mouse stained with anti-tdTomato showing free nerve endings just below the dermal surface, as well as lanceolate endings surrounding a hair follicle. Scale = 50 μ m. **(C)** An example of a lanceolate ending at a single hair, labeled by tdTomato (red) and co-stained with S100b (green); scale bar = 25 μ m. **(D)** Two-color fluorescent in situ hybridization using RNAscope in trigeminal ganglion sections from Trpv1^{lin}-tdT mice for a panel of sensory neuron markers. **(E)** Co-expression of Trpv1^{lin}-tdT with sensory neuron markers is quantified (see STAR Methods).

Figure S2. Related to Figure 2 and Figure 3; Heat maps of Calca-GCaMP6f

responses for all neurons imaged in the TG and DRG. (A) Raster plot showing that Calca cells are insensitive to stroking yet respond to hair pull and temperature (n=430; N=15). Note: the cells are ordered by size (largest to smallest, top to bottom). **(B)** A subset of Calca neurons responds robustly to heat. Only few cells have detectable calcium transients to warm temperatures (36°C) or to cooling (13°C). **(C)** Raster plots shows Calca neurons in the DRG that innervate hairy skin (hind leg) are responsive to high threshold mechanical stimulations (hair pull, pinch), and insensitive to lower threshold stimulations (stroking with and against the hair grain) (n=52; N=3). **(D)** Images of paw stimulation and DRG showing that after RTX treatment, mechanically-evoked responses persist on hairy but not glabrous skin (right).

Figure S3. Related to Figure 2 and Figure 3; Characterization of Calca-tdT

neurons. (A-B,D) Two color in situ hybridization with known markers of sensory cell types using RNAscope. **(A)** Small diameter Calca neurons (labeled with *tdTomato*, red) express *Trpv1* (green). **(B)** All neurons labeled with *tdTomato* (red) express *CGRP* (green). **(C)** Co-expression of *CGRP* with *tdTomato* is quantified. **(D)** Markers for different populations of sensory neurons (green) failed to label the medium diameter Calca neurons (*red*). **(E)** Co-expression of *tdTomato* with *Trpv1*, *Tac1*, *TrpA1*, and *Npy2R* are quantified (see STAR Methods).

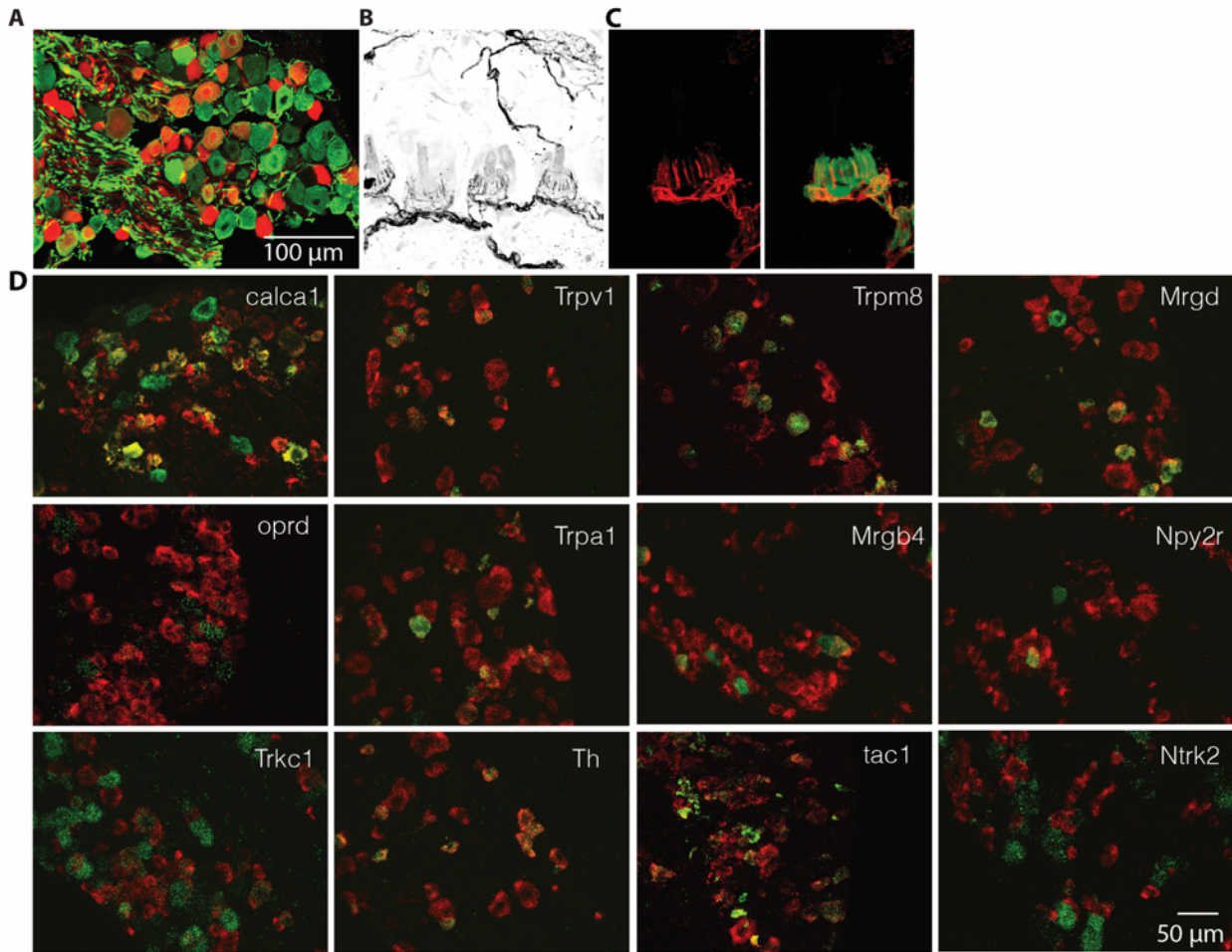
Figure S4: Related to Figure 3 and Figure 4; RTX treatment depleted *Trpv1*-positive, heat-responsive Calca neurons In-situ hybridization (RNA-scope, see methods) of TG (top panel) and DRG (bottom panel) in Calca-tdT mice showing *Trpv1* expression (green) is depleted with RTX treatment. Overlap of *Trpv1/Calca-tdT* is quantified (right of panel).

Figure S5. Related to Figure 3 and Figure 4; RTX treatment abolished small diameter neurons. Immunostaining of TG sections (top panel) and DRG (bottom panel) from Calca-tdT mice showing *tdTomato* (red) is found in sensory neurons with heterogeneous cell diameters. The largest neurons stained with *Nefh* (green) but do not project to superficial layers of skin (see Figure S6c); preliminary results indicate that these fibers innervate muscle. RTX treatment abolished the small diameter neurons (histograms on right).

Figure S6. Related to Figure 3; Calca-tdT neurons produce circumferential endings around hair shafts and free nerve endings in hairy skin independent of merkel cells. (A) Immunostaining for Troma-1 (green) shows Calca labeling is independent of merkel cells. (B) Images of hairy skin from the cheek (top) and belly (bottom) from Calca-tdT mice. Calca circumferential endings (arrow heads) wrap around hair shafts while free nerve endings (arrows) are found in more superficial layers. (C) Calca-tdT free nerve endings are NF200-negative.

Figure S7. Related to Figure 3 and Figure 4; Calca circumferential nerve endings in hairy skin persist following RTX treatment. (A) Representative confocal images from hairy skin (back), showing Calca-tdT nerve terminals. Following RTX treatment, circumferential nerve terminals around hair shafts largely persist, while free nerve endings in hairy skin and tail skin are ablated. (B-C) Quantification of hair shafts with circumferential endings (B), and lengths of free nerve endings per field of view (see STAR Methods) in hairy skin (C). Glabrous skin (D), and tail skin (E), before and following RTX treatment.

Figure S1



E

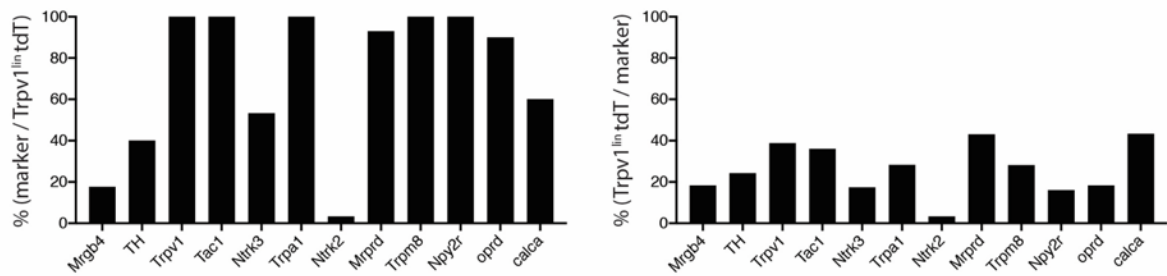


Figure S2

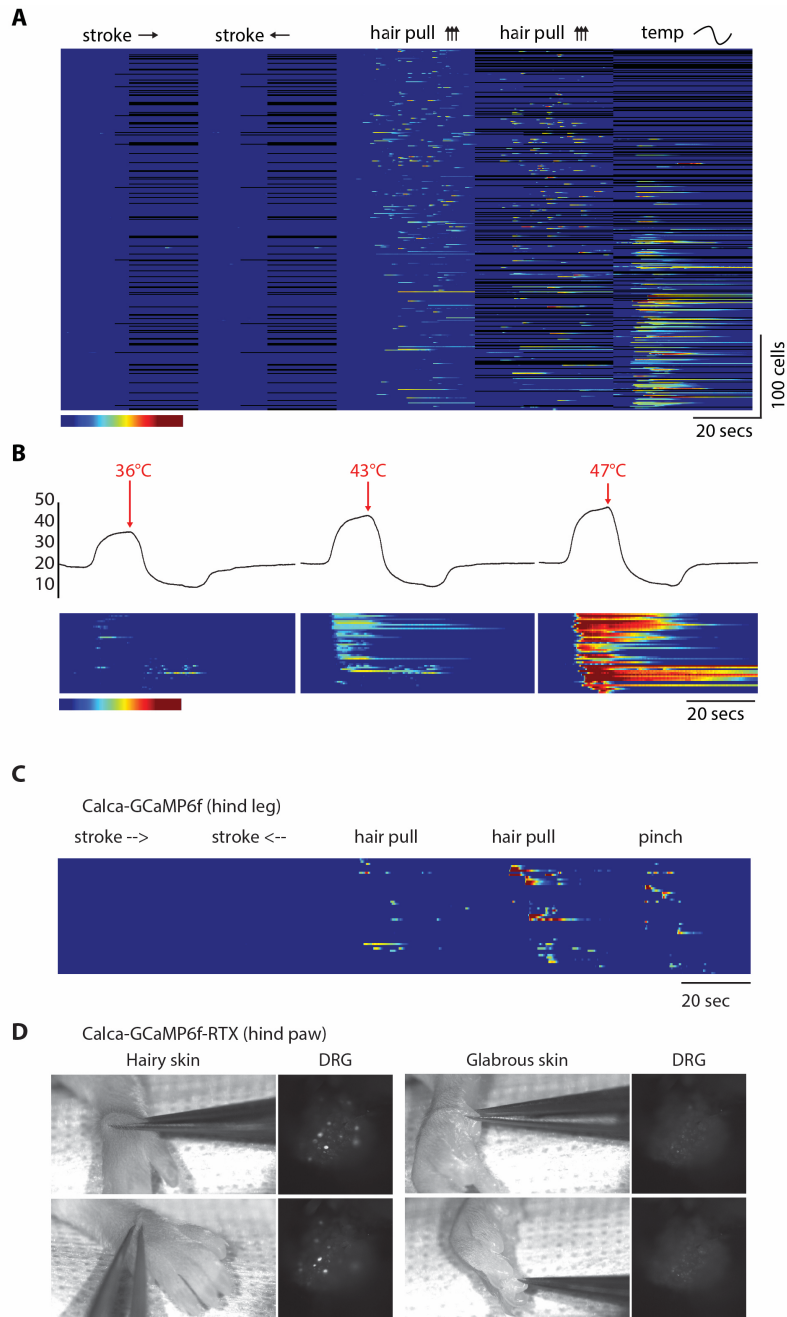


Figure S3

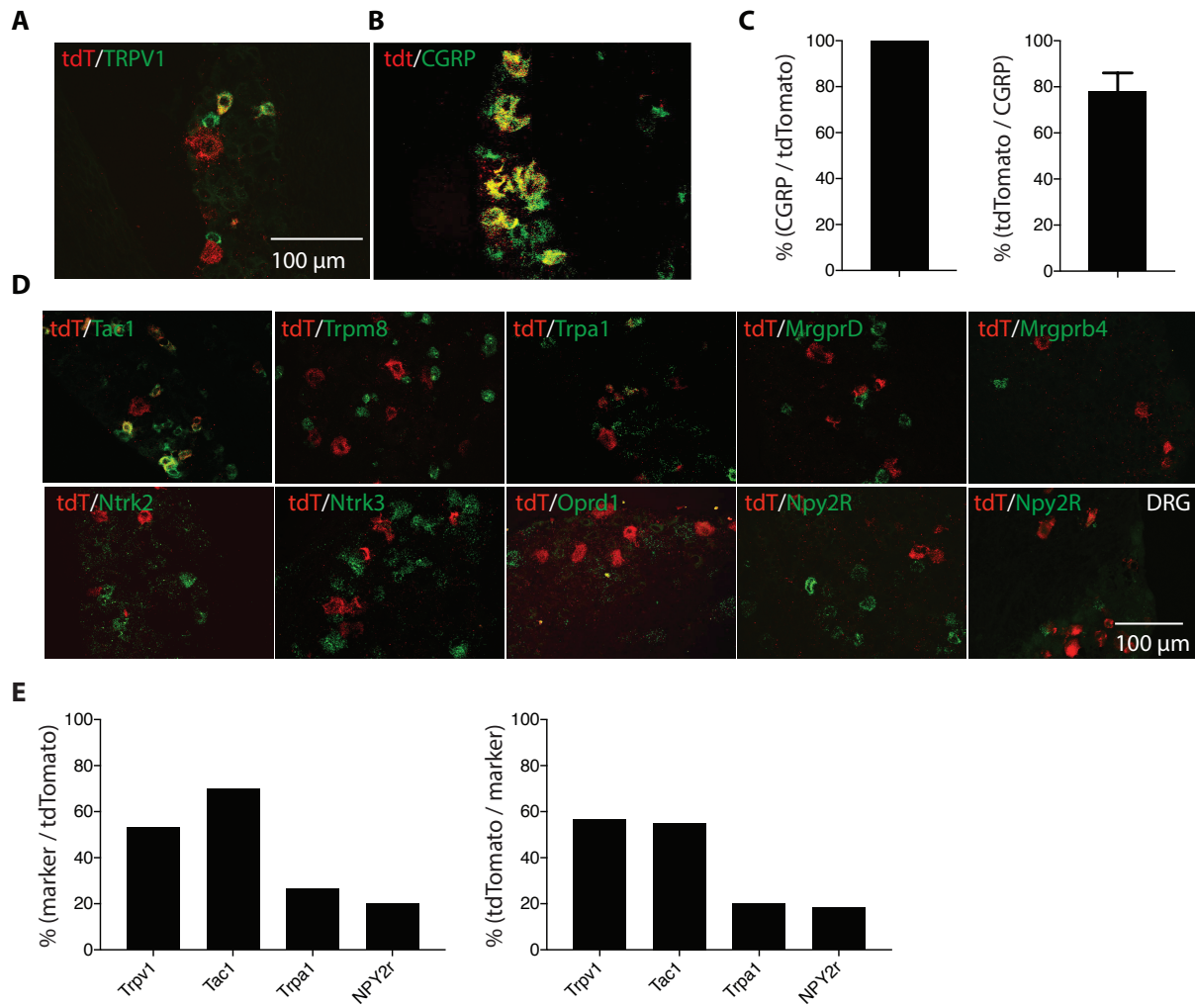


Figure S4

A

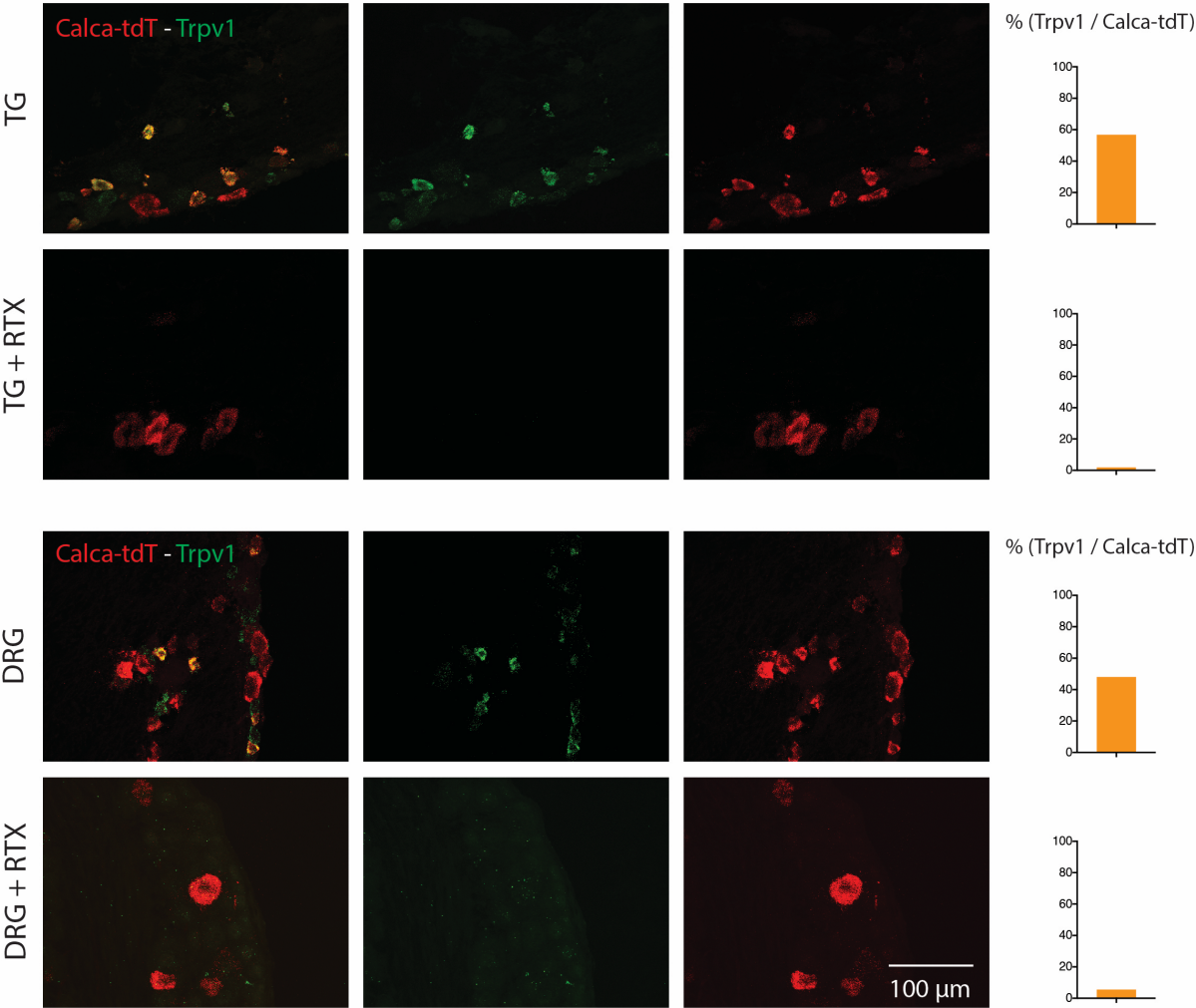


Figure S5

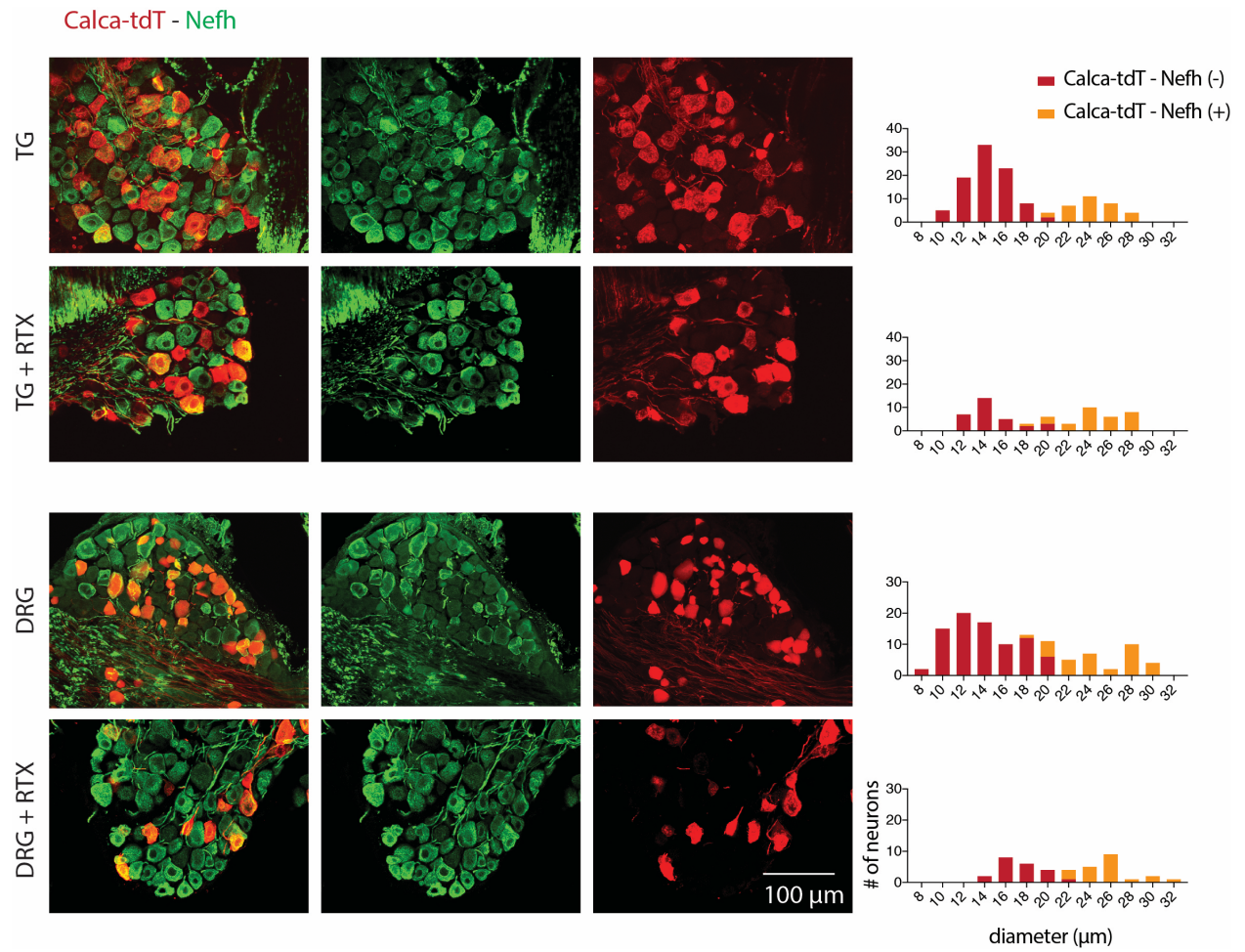
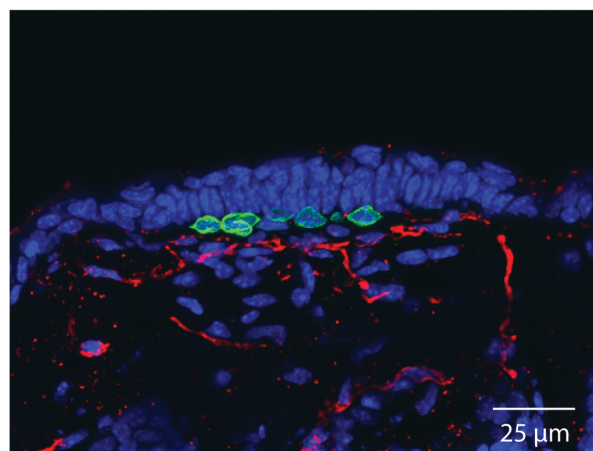


Figure S6

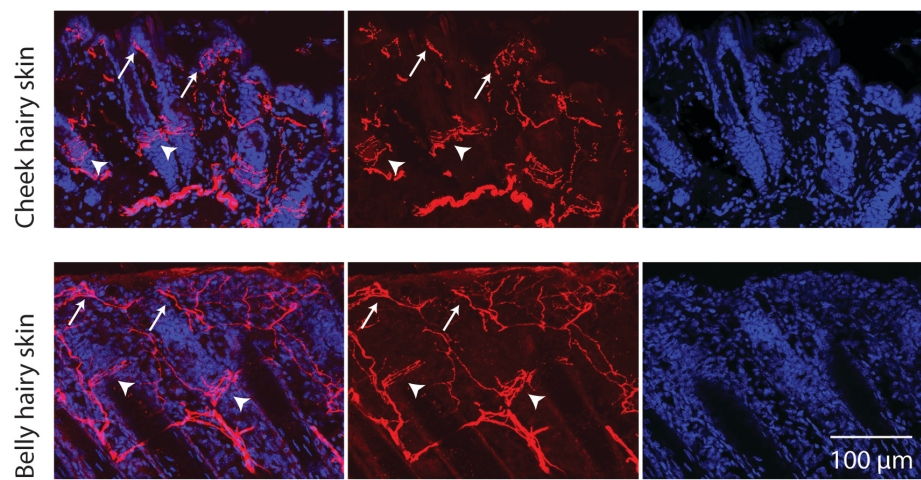
A

Troma1 - DAPI



B

Calca-tdT - DAPI



C

NF200 - DAPI

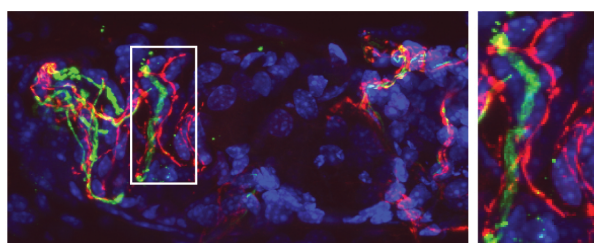


Figure S7

